

JAN 29 1997

To:

BRUESS, Steven C.  
MERCHANT, GOULD, SMITH, EDELL,  
WELTER & SCHMIDT  
3100 Norwest Center  
90 South Seventh Street  
Minneapolis, Minnesota 55402  
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF RECEIPT  
OF DEMAND

(PCT Rule 61.1(b), first sentence  
and Administrative Instructions, Section 601)

Date of mailing  
(day/month/year)

21. 01. 97

Applicant's or agent's file reference

600.311WOI1

IMPORTANT NOTIFICATION

International application No.

PCT/US 96/ 10252

International filing date (day/month/year)

07/06/1996

Priority date (day/month/year)

07/06/1995

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

03/01/1997

2. This date of receipt is:



the actual date of receipt of the demand.



the date on which the proper corrections to the demand were timely received.

3. ☐ This date is AFTER the expiration of 19 months from the priority date.

**Attention:** The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices)(Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.



This notification confirms the information given in person or by telephone on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA:



European Patent Office  
D-80298 Munich  
Tel. (+ 49-89) 2399-0, Tx: 523656 epmu d  
Fax: (+ 49-89) 2399-4465

Authorized officer

John Aitken

-27 35

Telephone No.

IPEA/ EP

# PCT

## CHAPTER II

### DEMAND

under Article 31 of the Patent Cooperation Treaty:  
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 600.311WO11
International application No. PCT/US96/10252	International filing date (day/month/year) 07 <sup>(07.06.96)</sup> June 1996	(Earliest) Priority date (day/month/year) 07 <sup>(07.06.95)</sup> June 1995
Title of invention MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) REGENTS OF THE UNIVERSITY OF MINNESOTA Morrill Hall, 100 Church Street S.E. Minneapolis, Minnesota 55455 United States of America		Telephone No.  Facsimile No.  Teleprinter No.
State (i.e. country) of nationality: US		State (i.e. country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) SCHLIEVERT, Patrick M. 5305 Birchcrest Drive Edina, Minnesota 55436 United States of America		
State (i.e. country) of nationality: US		State (i.e. country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ROGGIANI, Manuela 100 Second Street Southeast, #803 Minneapolis, Minnesota 55414 United States of America		
State (i.e. country) of nationality: IT		State (i.e. country) of residence: US
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.		

## Continuation of Box No. II APPLICANT(S)

*If none of the following sub-boxes is used, this sheet is not to be included in the demand.*

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

STOEHR, Jennifer  
3981 Woodridge Circle  
Vadnais Heights, Minnesota 55127  
United States of America

State (i.e. country) of nationality:

US

State (i.e. country) of residence:

US

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

OHLENDORF, Douglas  
9397 Olympia Drive  
Eden Prairie, Minnesota 55347  
United States of America

State (i.e. country) of nationality:

US

State (i.e. country) of residence:

US

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (i.e. country) of nationality:

State (i.e. country) of residence:

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (i.e. country) of nationality:

State (i.e. country) of residence:



Further applicants are indicated on another continuation sheet.

**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**The following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*BRUESS, Steven C.  
MERCHANT, GOULD, SMITH, EDELL,  
WELTER & SCHMIDT  
3100 Norwest Center  
90 South Seventh Street  
Minneapolis, Minnesota 55402  
United States of America

Telephone No.:

612/336-4711

Facsimile No.:

612/336-4751

Teleprinter No.:

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV STATEMENT CONCERNING AMENDMENTS**

The applicant wishes the International Preliminary Examining Authority\*

(i) ☐ to start the international preliminary examination on the basis of the international application as originally filed.(ii) ☒ to take into account the amendments under Article 34 of☒ the description (amendments attached).☒ the claims (amendments attached).☐ the drawings (amendments attached).(iii) ☐ to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).(iv) ☐ to disregard any amendments of the claims made under Article 19 and to consider them as reversed.(v) ☐ to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

- \* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

**Box No. V ELECTION OF STATES**☒ The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....*(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)*

## Box No. VI CHECKLIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:

- |                                                    |   |          |
|----------------------------------------------------|---|----------|
| 1. amendments under Article 34                     |   |          |
| description                                        | : | 2 sheets |
| claims                                             | : | 3 sheets |
| drawings                                           | : | sheets   |
| 2. letter accompanying amendments under Article 34 | : | 2 sheets |
| 3. copy of amendments under Article 19             | : | sheets   |
| 4. copy of statement under Article 19              | : | sheets   |
| 5. other (specify): <b>renumbered abstract</b>     | : | 1 sheets |

For International Preliminary  
Examining Authority use only

received

not received

☐☐☐☐☐☐☐☐☐☐☐☐☐☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature

4. ☒ fee calculation sheet

5. ☒ other (specify):

Acknowledgement Form (1 pg.)

## Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

By:   
Steven C. Bruess

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.

- ☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.


For International Bureau use only

Demand received from IPEA on:

## PCT

## FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No. <b>PCT/US96/10252</b>	For International Preliminary Examining Authority use only									
Applicant's or agent's file reference <b>600.311WO11</b>	Date stamp of the IPEA									
Applicant <b>REGENTS OF THE UNIVERSITY OF MINNESOTA et al.</b>										
<b>Calculation of prescribed fees</b>										
1. Preliminary examination fee .....	<b>3000 DM</b>	<input checked="" type="checkbox"/> P								
2. Handling fee ( <i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i> ) .....	<b>292 DM</b>	<input checked="" type="checkbox"/> H								
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box .....	<b>3292 DM</b> <b>TOTAL</b>									
<b>Mode of Payment</b>										
<table style="width: 100%;"> <tr> <td><input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below)</td> <td><input type="checkbox"/> cash</td> </tr> <tr> <td><input type="checkbox"/> cheque</td> <td><input type="checkbox"/> revenue stamps</td> </tr> <tr> <td><input type="checkbox"/> postal money order</td> <td><input type="checkbox"/> coupons</td> </tr> <tr> <td><input type="checkbox"/> bank draft</td> <td><input type="checkbox"/> other (specify):</td> </tr> </table>			<input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below)	<input type="checkbox"/> cash	<input type="checkbox"/> cheque	<input type="checkbox"/> revenue stamps	<input type="checkbox"/> postal money order	<input type="checkbox"/> coupons	<input type="checkbox"/> bank draft	<input type="checkbox"/> other (specify):
<input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below)	<input type="checkbox"/> cash									
<input type="checkbox"/> cheque	<input type="checkbox"/> revenue stamps									
<input type="checkbox"/> postal money order	<input type="checkbox"/> coupons									
<input type="checkbox"/> bank draft	<input type="checkbox"/> other (specify):									
<b>Deposit Account Authorization</b> ( <i>this mode of payment may not be available at all IPEAs</i> )										
The IPEA/ <u>EP</u> <input checked="" type="checkbox"/> is hereby authorized to charge the total fees indicated above to my deposit account.										
<input checked="" type="checkbox"/> ( <i>this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit</i> ) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.										
<b>2830 0082</b> Deposit Account Number	<b>03 January 1997</b> Date (day/month/year)	 Signature <b>Steven C. Bruess</b>								

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.  
Application Serial No. : PCT/US96/10252  
Filed : 07 June 1996  
Agent Ref. : 600.311WO11  
Title : MUTANTS OF STREPTOCOCCAL TOXIN A  
AND METHODS OF USE

**AMENDMENT UNDER ARTICLE 34 BEFORE THE  
PRELIMINARY EXAMINING AUTHORITY**

European Patent Office  
D-80298 Munchen  
GERMANY

Dear Sir:

Prior to Examination, Applicant requests the following amendments be made to the above-identified patent application.

**IN THE SPECIFICATION**

Please find enclosed specification pages 80a and 80b regarding Indications Relating to a Deposited Microorganism that are being submitted herewith under PCT Rule 13bis(a)(i) to (iii).

**IN THE CLAIMS**

Please amend the claims by substituting original claim pages 81 through 87 with new claim pages 81 through 83. (Please also find renumbered Abstract page 84.) Claims 1-39 are replaced by amended claims 1-16. Amended claims 1-16 correspond to the original claims as follows:

<b><u>New Claim</u></b>	<b><u>Original Claim</u></b>
1	1
2	1,5
3	1, 4, 5
4-9	4,5
10	2, 3, 5
11	6
12	15
13	19
14	20
15	22

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.  
 Application Serial No. : PCT/US96/10252  
 Filed : 07 June 1996  
 Agent Ref. : 600.311WOII  
 Title : MUTANTS OF STREPTOCOCCAL TOXIN A  
 AND METHODS OF USE

New Claim

16

canceled

Original Claim

22

7-14, 16-18, 21, 23-39

REMARKS

The claimed invention includes mutants of streptococcal pyrogenic exotoxin A (SPE-A). The mutants are substantially nonlethal compared to wild type SPE-A. Mutants can have amino acid substitutions in several secondary structure elements of SPE-A. In particular, mutants can have one to six amino acid substitutions compared with wild-type protein and at least one of the substituted amino acids is asparagine-20, lysine-157, cysteine-98 or cysteine-87.

The Hartwig et al. reference cited in the International Search Report discloses 9 mutants of SPE-A, each with a single amino acid substitution. Hartwig et al. does not disclose or suggest substitution for amino acids in the claimed secondary structural elements or residues. Furthermore, Hartwig neither discloses nor suggests mutants of SPE-A with more than one amino acid substitution. Therefore, Hartwig et al. neither teach nor suggest the claimed invention.

When the Examiner takes the present application up for examination, consideration of these amendments is respectfully requested, and issuance of a favorable written opinion is earnestly solicited.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,  
 WELTER & SCHMIDT, P.A.  
 3100 Norwest Center  
 90 South Seventh Street  
 Minneapolis, MN 55402  
 (612) 371-5278

Dated: 03 January 1997

By: 

Denise M. Kettelberger  
 Reg. No. 33,924



## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>35</u> . line S <u>8-11</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="checked" type="checkbox"/></span>	
Name of depositary institution <b>American Type Culture Collection</b>	
Address of depositary institution (including postal code and country) <b>12301 Parklawn Drive Rockville, MD 20852 United States of America</b>	
Date of deposit <b>(01.06.95) 01 June 1995</b>	Accession Number <b>69830</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: center; border-bottom: 1px solid black; margin-bottom: 5px;">For receiving Office use only</div> <div style="margin-bottom: 10px;"><input type="checkbox"/> This sheet was received with the international application</div> <div style="border: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="text-align: center; border-top: 1px solid black; margin-top: 5px;">Authorized officer</div>	<div style="text-align: center; border-bottom: 1px solid black; margin-bottom: 5px;">For International Bureau use only</div> <div style="margin-bottom: 10px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="text-align: center; border-top: 1px solid black; margin-top: 5px;">Authorized officer</div>
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# INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>44</u> . lines <u>9-10</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution  <p style="margin-left: 40px;">American Type Culture Collection</p>	
Address of depositary institution <i>(including postal code and country)</i>  <p style="margin-left: 40px;">12301 Parklawn Drive  Rockville, MD 20852  United States of America</p>	
Date of deposit <p style="margin-left: 40px;">(01.06.95) 01 June 1995</p>	Accession Number <p style="margin-left: 40px;">69831</p>
<b>C. ADDITIONAL INDICATIONS</b> <i>(leave blank if not applicable)</i> <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> <i>(if the indications are not for all designated States)</i>	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> <i>(leave blank if not applicable)</i>	
The indications listed below will be submitted to the International Bureau later <i>(specify the general nature of the indications e.g., "Accession Number of Deposit")</i>	

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<input type="checkbox"/> This sheet was received with the international application
Authorized officer

For International Bureau use only
<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer

**WHAT IS CLAIMED IS:**

1. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least one amino acid change and is  
5 substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

2. A mutant SPE-A toxin according to claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid  
10 substitutions; and

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A  
15 beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

3. A mutant SPE-A toxin according to claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid  
20 substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

4. The mutant SPE-A toxin of claim 3, wherein the at  
25 least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic  
30 acid.

5. The mutant SPE-A toxin of claim 4, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5

6. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

10 7. The mutant SPE-A toxin of claim 6, wherein the substitution is asparagine-20 to aspartic acid.

8. The mutant SPE-A toxin of claim 6, further comprising substitution of cysteine-98, or lysine-157.

15

9. The mutant SPE-A toxin of claim 8, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

20 10. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not  
25 lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

11. A vaccine for protecting animals against at least  
30 one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

12. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

5

13. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

14. A stably transformed host cell comprising a DNA  
10 sequence according to claim 13.

15. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 11 to an  
15 animal.

16. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 11 to an animal.

20

**ABSTRACT**

This invention is directed to mutant SPE-A toxins or fragments thereof, vaccine and pharmaceutical compositions, and methods of using the vaccine and pharmaceutical compositions. The preferred SPE-A toxin has at least one amino acid change and is substantially non-lethal compared with the wild type SPE-A toxin. The mutant SPE-A toxins can form vaccine compositions useful to protect animals against the biological activities of wild type SPE-A toxin.

# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum)

600.311WO11

Box No. I TITLE OF INVENTION

MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

REGENTS OF THE UNIVERSITY OF MINNESOTA  
Morrill Hall, 100 Church Street, Southeast  
Minneapolis, Minnesota 55455  
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (i.e. country) of nationality:

US

State (i.e. country) of residence:

US

This person is applicant  
for the purposes of:



all designated  
States



all designated States except  
the United States of America



the United States  
of America only



the States indicated in  
the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

SCHLIEVERT, Patrick M.  
5305 Birchcrest Drive  
Edina, Minnesota 55436  
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box  
is marked, do not fill in below.)

State (i.e. country) of nationality: US

State (i.e. country) of residence: US

This person is applicant  
for the purposes of:



all designated  
States



all designated States except  
the United States of America



the United States  
of America only



the States indicated in  
the Supplemental Box



Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf  
of the applicant(s) before the competent International Authorities as:



agent



common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

BRUESS, Steven C.  
MERCHANT, GOULD, SMITH, EDELL,  
WELTER & SCHMIDT, P.A.  
3100 Norwest Center  
90 South Seventh Street  
Minneapolis, Minnesota 55402

Telephone No.

612/336-4711

Facsimile No.

612/336-4751

Teleprinter No.

United States of America

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

## Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

*If none of the following sub-boxes is used, this sheet is not to be included in the request.*

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

ROGGIANI, Manuela  
100 Second Street Southeast, #803  
Minneapolis, Minnesota 55414  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

IT

State (i.e. country) of residence:

US

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

STOEHR, Jennifer  
3981 Woodridge Circle  
Vadnais Heights, Minnesota 55127  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

US

State (i.e. country) of residence:

US

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

OHLENDORF, Douglas  
9397 Olympia Drive  
Eden Prairie, Minnesota 55347  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

US

State (i.e. country) of residence:

US

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.



**Box No.V DESIGNATION OF STATES**

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes: at least one must be marked):

**Regional Patent**

- ☒ AP ARIPO Patent: KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AZ Azerbaijan, BY Belarus, KZ Kazakstan, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT, AM Armenia, KG Kyrgyzstan, MD Republic of Moldova
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☒ CIP Application

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In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of \_\_\_\_\_

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:

in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part";
- (vi) if there are more than three earlier applications whose priority is claimed;

in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;

in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;

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in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:

in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.

#### CONTINUATION OF BOX V:

US which is a Continuation-In-Part of 08/480,261 filed 07 June 1995.

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) US	07 June 1995 (07.06.95)	08/480,261	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : (1)

Box No. VII **INTERNATIONAL SEARCHING AUTHORITY**

**Choice of International Searching Authority (ISA)** (If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen: the two-letter code may be used): ISA / EP

**Earlier search** Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.

Country (or regional Office): Date (day/month/year): Number:

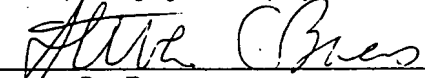
Box No. VIII **CHECK LIST**

<p>This international application contains the following number of sheets:</p> <p>1. request : 5 sheets</p> <p>2. description : 80 sheets</p> <p>3. claims : 7 sheets</p> <p>4. abstract : 1 sheets</p> <p>5. drawings : 9 sheets</p> <p>Total : 102 sheets</p>	<p>This international application is accompanied by the item(s) marked below:</p> <p>1. <input type="checkbox"/> separate signed power of attorney</p> <p>2. <input checked="" type="checkbox"/> copy of general power of attorney</p> <p>3. <input type="checkbox"/> statement explaining lack of signature</p> <p>4. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s):</p> <p>5. <input checked="" type="checkbox"/> fee calculation sheet</p> <p>6. <input checked="" type="checkbox"/> separate indications concerning deposited microorganisms</p> <p>7. <input checked="" type="checkbox"/> nucleotide and/or amino acid sequence listing (diskette)</p> <p>8. <input checked="" type="checkbox"/> other (specify): PCT Transmittal Gen. Transmittal (in dupl.)</p> <p>X Return Postcard Check in amount \$5352.00</p>
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Figure No. \_\_\_\_\_ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX **SIGNATURE OF APPLICANT OR AGENT**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

BY:   
Steven C. Bruess

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3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Patrick M. SCHLIEVERT, et al. Examiner: Unassigned  
Serial No.: Unassigned Group Art Unit: Unassigned  
(Based on PCT/US96/10252)

Filed: Concurrently herewith Docket No.: 600.311USWO  
(International Filing Date of 07 June 1996)

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Title: MUTANTS OF STREPTOCOCCAL TOXIN A AND METHODS OF USE

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CERTIFICATE UNDER 37 CFR 1.10

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I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By: 

Name: William Smith

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Dear Sir:

IN THE ABSTRACT

Please insert the attached Abstract page (page 85) into the application as the last page thereof.

IN THE SPECIFICATION

Page 1, after the title, please insert the following paragraph:

--This application is a Continuation-In-Part of application Serial No.

08/480,261, filed June 7, 1995, now abandoned.--

Please amend the specification to include specification pages 80a and 80b filed under Article 34 with the filing of the Demand for Examination on January 3, 1997. A copy of pages 80a and 80b are enclosed.

Please also amend the specification to replace originally filed page 3 with specification pages 3 and 3a (copies enclosed) filed in Response to the Written Opinion on

August 1, 1997, and included with the Notification of Transmittal of International Preliminary Examination Report mailed September 19, 1997.

### IN THE CLAIMS

Please replace the claims with the claims (amended claims 1-23) filed in Response to the Written Opinion on August 1, 1997 (copies enclosed), and included with the Notification of Transmittal of International Preliminary Examination Report mailed September 19, 1997.

### REMARKS

A new Abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

A courtesy copy of the Notification of Transmittal of International Preliminary Examination Report is also enclosed.

The claims have been amended to bring the application into conformance with the PCT Chapter II application as amended after publication. It should be noted that the filing fees provided in connection with the application filed herewith are based on the entry of this Preliminary Amendment.

Respectfully submitted,

PATRICK M. SCHLIEVERT et al.,

By their attorneys,  
MERCHANT, GOULD, SMITH, EDELL,  
WELTER & SCHMIDT, P.A.  
3100 Norwest Center  
Minneapolis, Minnesota 55402  
(612) 332-5300

Date December 5, 1997

By Steven C. Bruess  
Steven C. Bruess  
Reg. No. 34,130

staphylococcal toxic shock syndrome toxin 1,  
staphylococcal enterotoxins A, B, Cn, D, E, G and H,  
and non-group A streptococcal pyrogenic exotoxins.

- 5 These toxins have similar biochemical properties,  
biological activities and various degrees of sequence  
similarity.

The most severe manifestations of STSS are  
hypotension and shock, that lead to death. It is  
10 generally believed that leakage of fluid from the  
intravascular to the interstitial space is the final  
cause of hypotension, supported by the observation that  
fluid replacement therapy is successful in preventing  
shock in the rabbit model of STSS described above. It  
15 has been hypothesized that SPE-A may act in several ways  
on the host to induce this pathology. Certain single  
amino acid substitutions in central regions of the SPE-A  
molecule have been shown to affect the mitogenic  
activity of and binding to a HLA class II molecules by  
20 SPE-A (Hartwig et al. International Immunology 5:5, 869-  
875 (1993)).

SPE-A has been shown to block liver clearance of  
endotoxin of endogenous flora's origin, by comprising  
the activity of liver Kupffer cells. This appears to  
25 cause a significant increase in circulating endotoxin,  
that through binding to lipopolysaccharide binding  
protein (LBP) and CD14 signaling leads to macrophage  
activation with subsequent release of TNF- $\alpha$  and other  
cytokines. Support for the role of endotoxin in the  
30 disease is given by the finding that the lethal effects  
of SPE-A can be at least partially neutralized by the

administration to animals of polymyxin B or by the use of pathogen free rabbits.

Another modality of induction of shock could be the  
5 direct activity of the toxin on capillary endothelial cells. This hypothesis stems from the finding that the staphylococcal pyrogenic toxin TSST-1 binds directly to the human umbilical cord vein cells and is cytotoxic to isolated porcine aortic endothelial cells.

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>35</u> . line S <u>8-11</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input checked="checked" type="checkbox"/></span>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>12301 Parklawn Drive</u> <u>Rockville, MD 20852</u> <u>United States of America</u>	
Date of deposit <u>(01.06.95) 01 June 1995</u>	Accession Number <u>69830</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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Address of depositary institution (including postal code and country)  <u>12301 Parklawn Drive</u> <u>Rockville, MD 20852</u> <u>United States of America</u>	
Date of deposit  <u>(01.06.95) 01 June 1995</u>	Accession Number  <u>69831</u>
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WHAT IS CLAIMED IS:

1. A mutant SPE-A toxin or fragment thereof, the mutant SPE-A toxin comprising one to six amino acid substitutions and being substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin;

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

2. The mutant SPE-A toxin of claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

20

3. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

4. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

5

6. The mutant SPE-A toxin of claim 5, wherein the substitution is asparagine-20 to aspartic acid.

10

7. The mutant SPE-A toxin of claim 5, further comprising substitution of cysteine-98, or lysine-157.

15

8. The mutant SPE-A toxin of claim 7, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

20

9. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

25

10. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

30

11. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

5 12. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

13. A stably transformed host cell comprising a DNA sequence according to claim 12.

10

14. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 10 to an animal.

15

15. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 10 to an animal.

20 16. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least two amino acid changes and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

25

17. The mutant SPE-A toxin of claim 16, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not  
30 substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains

mitogenicity comparable to that of the wild type SPE-A toxin.

18. A vaccine for protecting animals  
5 against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 16.

19. A pharmaceutical composition  
10 comprising: a mutant SPE-A according to claim 16 in admixture with a physiologically acceptable carrier.

20. A DNA sequence encoding a mutant SPE-A toxin according to claim 16.  
15

21. A stably transformed host cell comprising a DNA sequence according to claim 29.

22. A method for protecting an animal  
20 against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 18 to an animal.

23. A method for reducing symptoms  
25 associated with toxic shock comprising: administering a vaccine according to claim 18 to an animal.

**ABSTRACT**

This invention is directed to mutant SPE-A toxins or fragments thereof, vaccine and pharmaceutical compositions, and methods of using the vaccine and pharmaceutical  
5 compositions. The preferred SPE-A toxin has at least one amino acid change and is substantially non-lethal compared with the wild type SPE-A toxin. The mutant SPE-A toxins can form vaccine compositions useful to protect animals against the biological activities of wild type SPE-A toxin.